

1 **Effect of PEEP, blood volume, and inspiratory hold maneuvers on venous**  
 2 **return**

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26 **Running head:** Venous Return, Blood Volume and Ventilatory Maneuvers

27

28 **Abstract**

29

30 According to Guyton's model of circulation, mean systemic filling pressure (MSFP),  
31 right atrial pressure (RAP), and resistance to venous return (RVR) determine venous  
32 return. MSFP has been estimated from inspiratory hold-induced changes in RAP and  
33 blood flow. We studied the impact of positive end expiratory pressure (PEEP) and  
34 blood volume on venous return and MSFP in pigs.  $MSFP_{RAO}$  was measured by  
35 balloon occlusion of right atrium and  $MSFP_{insp\_hold}$  extrapolated from RAP/pulmonary  
36 artery flow ( $Q_{PA}$ ) relationships during inspiratory holds at PEEP 5 and 10 cmH<sub>2</sub>O,  
37 after bleeding and in hypervolemia.  $MSFP_{RAO}$  increased with PEEP [PEEP 5, mean  
38 (SD) 12.9 (2.5) mmHg; PEEP 10 14.0 (2.6) mmHg,  $p=.002$ ] without change in  $Q_{PA}$   
39 [2.75 (.43) vs. 2.56 (.45) L/min,  $p=.094$ ].  $MSFP_{RAO}$  decreased after bleeding and  
40 increased in hypervolemia [10.8 (2.2) and 16.4 (3.0) mmHg respectively  $p<.001$ ], with  
41 parallel changes in  $Q_{PA}$ . Neither PEEP nor volume state altered RVR ( $p=.489$ ).  
42  $MSFP_{insp\_hold}$  overestimated  $MSFP_{RAO}$  [16.5 (5.8) mmHg vs. 13.6 (3.2) mmHg;  $p=.001$ ;  
43 mean difference 3.0 (5.1) mmHg]. Inspiratory holds shifted the RAP/ $Q_{PA}$  relationship  
44 rightwards in euolemia because inferior vena cava flow ( $Q_{IVC}$ ) recovered early after  
45 an inspiratory hold nadir. The  $Q_{IVC}$  nadir was lowest after bleeding [36 % (24 %) of  
46 pre-inspiratory hold at 15 cmH<sub>2</sub>O inspiratory pressure] and the  $Q_{IVC}$  recovery most  
47 complete at lowest inspiratory pressures independent of volume state [range from 80  
48 (7) % after bleeding to 103 (8) % at PEEP 10 cmH<sub>2</sub>O of  $Q_{IVC}$  before inspiratory hold].  
49 The  $Q_{IVC}$  recovery thus defends venous return, possibly via hepatosplanchnic  
50 vascular waterfall.

51

52 **New and Noteworthy:**

53 Enhanced recovery of  $Q_{IVC}$  during inspiratory holds shifts the RAP/ $Q_{PA}$  relationship to  
54 the right. Hence,  $MSFP_{insp\_hold}$  overestimates the  $MSFP_{RAO}$ . The preferential  $Q_{IVC}$   
55 recovery helps to maintain venous return during sustained increased inspiratory  
56 airway pressure. The underlying mechanism is likely to be a hepatosplanchnic  
57 vascular waterfall.

58

59 **Keywords:** right atrial pressure, mean systemic filling pressure, mechanical  
60 ventilation, blood volume, cardiac output

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62 on Intensive Care and Emergency Medicine, 15th to 18th March 2016, in Brussels.

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64

## 65    **Introduction**

66    Positive pressure ventilation has complex cardiovascular effects, which often  
67    necessitate administration of fluids or vasoactive drugs to support hemodynamics.  
68    Changes in hemodynamic measurements during the ventilator cycle have been  
69    proposed as a means to assess the potential response of cardiovascular system to  
70    fluids (45, 48, 49, 58).

71

72    The effects of positive pressure ventilation and application of positive end expiratory  
73    pressure on cardiac output can be explained by the interactions of the venous return  
74    function and cardiac function (19) – a concept proposed by Guyton more than 50  
75    years ago (24). The effects of positive intrathoracic pressures on cardiac function  
76    have been well elucidated in patients with respiratory and circulatory failure (11, 59).  
77    In contrast, the effects of mechanical ventilation on the venous return function are  
78    more difficult to evaluate due to lack of clinically available methods to assess its  
79    variables.

80    The total blood volume consists of unstressed and stressed volume. The unstressed  
81    volume fills the vasculature without pressurizing, whereas the stressed volume  
82    causes elastic recoil pressure(47). The mean systemic filling pressure is the elastic  
83    recoil pressure caused by the stressed volume in the systemic circulation. It can be  
84    quantified during an acute no flow state (28). Venous return according to Guyton's  
85    model is driven by the gradient between MSFP and right atrial pressure. Thus, at  
86    zero blood flow the RAP equals the MSFP. When the rate of venous return is plotted  
87    as a function of RAP, it follows a linear function and the slope of the curve is the  
88    inverse of resistance to venous return, RVR (24, 27, 39, 60). The RVR reflects the



89 composite resistance of all systemic vascular beds for the blood flow returning to the  
90 heart(25, 68).

91 In Guyton's model, the working heart serves dual roles. It lowers RAP and thereby  
92 enables venous return and it provides the mechanical energy that maintains driving  
93 pressure for peripheral tissue perfusion (44, 46). Even though Guyton's model of the  
94 circulation is heavily criticized (2, 3, 36) and debated (6, 7, 44, 46, 56), approaches  
95 based on this concept have gained renewed interest for explaining hemodynamic  
96 instability and planning therapeutic interventions (19, 31, 43, 55, 70, 71). Specifically,  
97 changes in MSFP could help to assess changes in stressed volume.

98 Since MSFP cannot be directly measured in clinical practice, surrogate approaches  
99 have been proposed (42). These include extrapolation from pressure/flow  
100 relationships during inspiratory hold maneuvers (27, 39, 60), extrapolation from  
101 peripheral venous and arterial pressures during instantaneous vascular occlusion  
102 (21), and mathematical modeling (12, 54, 55). However, an important limitation of the  
103 interventional methods used to estimate MSFP is that they may trigger vascular  
104 reflexes and other adaptive responses that can alter MSFP and RVR. These  
105 approaches assume that Guyton's model for steady state conditions would be  
106 applicable in the presence of transient changes in pressures and flow – an  
107 assumption that has not been validated.

108

109 We used a porcine model to address the following questions: 1) Do changes in  
110 PEEP, volume status and tidal breaths alter MSFP and the slope of the venous return  
111 curve? 2) Does a measurement of MSFP obtained with inspiratory hold maneuvers  
112 correspond to MSFP measured by right atrial occlusion? 3) Do inspiratory hold

113 maneuvers modify the hemodynamic variables of the venous return function, and do  
114 PEEP and volume status modify these responses? The answers to these questions  
115 have important implications for the attempts by investigators to use respiratory  
116 maneuvers to assess MSFP.

117

## 118 **Glossary**

119

120	$C_{\text{vascular}}$	Compliance of the vascular system
121	$F_{\text{I}O_2}$	Fraction of inspired oxygen
122	HES	Hydroxyethyl starch
123	IVC	Inferior vena cava
124	MAP	Mean arterial pressure
125	MSFP	Mean systemic filling pressure
126	$MSFP_{\text{insp\_hold}}$	Mean systemic filling pressure obtained via extrapolation of
127		pressure-flow relationships with airway occlusion
128	$MSFP_{\text{RAO}}$	mean systemic filling pressure; measured during right atrial
129		balloon occlusion at end expiratory lung volume
130	PA	Pulmonary artery
131	$P_{\text{AW}}$	Airway pressure
132	PAP	Pulmonary artery pressure
133	$P_{\text{insp}}$	Inspiratory airway pressure
134	PEEP	Positive endexpiratory pressure
135	$Q_{\text{PA}}$	Pulmonary artery blood flow
136	$Q_{\text{IVC}}$	Inferior vena cava blood flow
137	$Q_{\text{SVC}}$	Superior vena cava blood flow
138	RA	Right atrium
139	RAP	Right atrial pressure

140	RAP <sub>tm</sub>	Right atrial transmural pressure
141	RVR	Resistance to venous return
142	SVC	Superior vena cava
143	TV	Tidal ventilation
144	VRdP	Venous return driving pressure
145	V <sub>s</sub>	Stressed volume
146	V <sub>u</sub>	Unstressed volume

147

## 148 **Materials and methods**

149 The study complied with the Guide for the Care and Use of Laboratory Animals,  
150 National Academy of Sciences 1996, and Swiss National Guidelines and was  
151 approved by the Commission of Animal Experimentation of Canton Bern, Switzerland  
152 (approval number BE 71/14). Twelve domestic male pigs [body weight 39.1 (SD 1.7)]  
153 kg were fasted for 12 hours with free access to water. The first two pigs were used in  
154 pilot studies to establish the instrumentation and the feasibility of the study  
155 procedures. Ten pigs were included in the study. After premedication with  
156 intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg) anesthesia was induced  
157 with midazolam (0.5 mg/kg) and the pigs were orally intubated. Anesthesia was  
158 maintained with propofol (4 mg/kg/h) and fentanyl (5 µg/kg/h) and the depth  
159 controlled by repeatedly testing the response to nose pinch. Additional injections of  
160 fentanyl (50 µg) or midazolam (5 mg) were given as needed. Muscle relaxation was  
161 induced with rocuronium (0.5 mg/kg) for the study measurements. The pigs were  
162 mechanically ventilated in a volume controlled mode (Servo-I, Maquet Critical Care,  
163 Solna, Sweden) using positive end-expiratory pressure of 5 cm H<sub>2</sub>O, a F<sub>I</sub>O<sub>2</sub> of .30,

and a tidal volume of 300 mL [7.7 (0.3) mL/kg body weight]. Respiratory rate was adjusted to maintain an end-tidal pCO<sub>2</sub> of 40 mmHg.

### *Installations*

The following catheters were surgically placed for the measurements of arterial and venous pressures: two double-lumen catheters in the superior vena cava via the right and left jugular vein, a catheter in the right carotid artery, an arterial and a venous catheter in the right hind limb, and introducer sheaths in the right and left femoral veins. A median sternotomy was used to enter the thoracic cavity. The pericardium was opened and appropriately sized transit time ultrasonic flow probes (Transonic Systems, Inc., Ithaca, NY, USA) were placed around the main pulmonary artery, the superior vena cava and the inferior vena cava. Another catheter was placed in the main trunk of the PA and a 12×20 mm balloon catheter for measurement of pericardial pressure (Tyshak II, Numed, Canada) was fixed in the pericardium at the level of the right atrium (35). All catheters and cables were guided outside the thoracic cavity. The pleural cavities were drained and placed under pressure of minus 20 cm H<sub>2</sub>O until the measurements were started. The pericardium was closed by a continuous mattress suture, the sternum with figure of eight sutures, and the wound in layers. The urinary bladder was drained via a cystostomy. An esophageal balloon catheter (Sidam, Mirandola, Italy) was orally inserted to estimate changes in pleural pressure (14). The position of the pericardial and esophageal balloons was confirmed by chest compression during an expiratory hold (61). A catheter with a 50 mm×34 mm inflatable high compliance balloon (Amplatzer sizing balloon, St. Jude Medical, St. Paul, MN, USA) was introduced under fluoroscopy through the femoral vein sheath into the RA and a multilumen catheter was placed in the IVC. The position of the RA balloon and the catheters for measurement of pressure in the SVC and IVC

(both placed intrathoracically), as well as the location of the RA for zero reference of intravascular pressures were confirmed by fluoroscopy.

During surgery, Ringer's lactate was infused at a rate of 10 mL/kg/h, and in case of relevant blood loss supplemented by boluses of Ringer's lactate or hydroxyethyl starch (6% Voluven; Fresenius Kabi, Bad Homburg, Germany). After surgery the infusion rate was 3 mL/kg/h. Antibiotic prophylaxis was given as 1.5 g cefuroxime at skin incision and 4 hours later. Non-fractionated heparin was infused at a rate of 10'000 units/24 hours as thrombosis prophylaxis.

#### *Data acquisition*

Intravascular (carotid artery, PA, RA, SVC and IVC), esophageal, pericardial and airway pressures were measured using transducers (xtrans®, Codan Medical, Germany) and a multi-modular patient monitor (S/5 Critical Care Monitor®; Datex-Ohmeda, GE Healthcare, Helsinki, Finland), which also provided continuous ECG, end-tidal pCO<sub>2</sub> and body temperature. All pressure signals and the ultrasonic blood flow signals were recorded at 100 Hz in a data acquisition system (Labview™; National Instruments Corp., Austin, TX, USA), and processed off line using a customized analysis software (Soleasy, Alea Solutions, Zürich, Switzerland). The pressure transducers were calibrated using a water scale and the flow transducers zeroed and calibrated electronically before the study measurements. Baseline drift was checked, including zero flow in vivo, at the end of the experiment.

After surgery, 90 minutes were allowed for stabilization. Then, two 100 mL boluses of HES were given to replace any potential remaining perioperative volume deficit, and

in case of a stroke volume increase of  $>10\%$ , one further bolus was given. In the first animal Ringer's lactate was given instead of HES. After the volume boluses, baseline hemodynamics were recorded at PEEP 5 cm H<sub>2</sub>O.

### *Study protocol*

The protocol consisted of a series of five experimental conditions, at which the variables of the venous return function were individually assessed. In the first two conditions PEEP 5 cm H<sub>2</sub>O and PEEP 10 cm H<sub>2</sub>O were applied in random order. Three volume states followed at PEEP 5 cm H<sub>2</sub>O. The volume states started with *euvolemia* followed by stepwise *bleeding* (6 and 3 mL/kg body weight) and *hypervolemia* after rapid retransfusion of twice the bled volume with the shed heparinized blood diluted in 1:1 with HES.

In each condition, MSFP was assessed during a circulatory arrest induced by balloon occlusion of the right atrium (MSFP<sub>RAO</sub>) and extrapolated from inspiratory hold maneuvers (MSFP<sub>insp\_hold</sub>). Detailed descriptions are given below. The order of MSFP<sub>RAO</sub> and MSFP<sub>insp\_hold</sub> maneuvers was randomized using opaque sealed envelopes. A graphical summary of the protocol is given in Figure 1. As in steady state conditions, pulmonary artery blood flow and cardiac output are essentially the same, we use Q<sub>PA</sub> and cardiac output interchangeably.

### *MSFP<sub>RAO</sub>*

To measure the MSFP<sub>RAO</sub> a right atrial balloon was rapidly filled under fluoroscopic control with a mixture of radiocontrast and saline for 60 seconds at end expiratory lung volume. PA pressure and flow tracings confirmed circulatory arrest. MSFP<sub>RAO</sub>

was estimated as mean value of SVC and IVC pressure curves for 3 seconds as they approached a plateau at 9-12 seconds of RA occlusion before the onset of sympathetic reflex vasoconstriction, which was identified as a further increase in all intravascular pressures (Figure 2). The  $MSFP_{RAO}$  was considered as the reference for true MSFP and was therefore used as the upstream pressure in all calculations of resistance to venous return unless indicated otherwise. Similar approaches have been used by others (51).

#### *Total blood volume, stressed and unstressed volume and vascular compliance*

Blood volume was measured using indocyanine green dye dilution (29) during baseline conditions at PEEP 5 cm H<sub>2</sub>O, during *euvolemia* before *bleeding*, and in *hypervolemia* after retransfusion (Figure 1). The plasma dye concentration was measured by spectrophotometry. Ten blood samples were taken at 20 seconds intervals starting at 120 seconds after a bolus injection. The dye disappearance rate from plasma was extrapolated to time zero to calculate the plasma volume, and the blood volume using the mean hematocrit of an arterial and venous blood sample.

The blood volume measured at *euvolemia* before the bleeding and the rapid blood volume changes (*bleeding*, *hypervolemia* after retransfusion of blood and HES) were used to plot MSFP as a function of blood volume and to calculate the corresponding linear regression. The intercept at zero MSFP represents the unstressed volume ( $V_u$ ), and the slope of the linear regression line the inverse of vascular compliance ( $C_{vascular}$ ). Assuming linear compliance (15, 40, 53, 67) across the blood volumes measured, the stressed volume corresponding to the  $MSFP_{RAO}$  could be calculated (15, 40, 76) (Figure 3).

### *Reference function for venous return*

The reference venous return function was constructed with the mean RAP and  $Q_{PA}$  of ten heart cycles during tidal ventilation immediately preceding the balloon occlusion, and the  $MSFP_{RAO}$ . VRdP was calculated for each pig and experimental condition as  $MSFP_{RAO}-RAP$  and RVR as  $(MSFP_{RAO}-RAP)/Q_{PA}$ . Thus, RVR is equal to the inverse of the slope of a line connecting  $Q_{PA}$  and RAP before the atrial balloon occlusion and the subsequent  $MSFP_{RAO}$ .

### *Extrapolation of $MSFP_{insp\_hold}$ with inspiratory hold maneuvers*

Expiratory and inspiratory hold maneuvers at the respective PEEP and plateau pressures of 15, 20, 25 and 30 cm H<sub>2</sub>O were done by adjusting tidal volume. Accordingly, the difference between inspiratory hold pressures and PEEP was smaller at PEEP 10 cm H<sub>2</sub>O when compared to the other conditions.

$Q_{PA}$  and RAP were taken as mean values over three cardiac cycles after 9 seconds of each expiratory and inspiratory hold.  $MSFP_{insp\_hold}$  was defined as the zero flow intercept extrapolated from the plot of  $Q_{PA}$  as a function of RAP at these different airway pressures (Figure 4) (27, 38-42). A goodness of fit  $r^2 > 0.7$  was considered as prerequisite for inclusion in analysis.

### *Effect of tidal breathing*

The impact of changing from tidal ventilation to expiratory hold was assessed using the mean RAP and  $Q_{PA}$  of ten heart cycles during tidal ventilation preceding the expiratory hold and from the beginning of the expiratory hold.



### *Flow behavior in the thoracic veins*

The impact of the inspiratory holds on SVC and IVC blood flows was evaluated. The relative decreases in SVC and IVC blood flows to the nadir beat of each flow during the inspiratory hold were compared to the values of a tidal breath preceding the respective inspiratory hold. Similarly, the three cardiac cycles used to calculate the  $MSFP_{insp\_hold}$  during the inspiratory holds were compared to those during the tidal breath preceding the inspiratory holds in order to document flow recovery from the nadir during the inspiratory hold (Figure 4 Panel C).

### *Transmural pressures of the SVC and right atrium*

Transmural pressure was calculated as intravascular minus esophageal pressure for the SVC and as RAP minus pericardial pressure for the right atrium (35), respectively. We report differences in transmural pressure between experimental conditions and changes from inspiration to expiration during the airway maneuvers. We have used this approach previously (5), as absolute esophageal pressures are less reliable than their changes from inspiration to expiration (22, 23).

### *Statistical analysis*

Data were analyzed using SPSS software (Version 21; SPSS Inc., Chicago Illinois, USA). Paired t-test (for the two PEEP levels) and analysis of variance for repeated measures (for the three volume states) were used to analyze hemodynamics during tidal ventilation and at end expiratory lung volume. Analysis of variance for repeated measures was used to compare  $MSFP_{RAO}$  and  $MSFP_{insp\_hold}$  (within subject factor method, grouping factor experimental condition), venous return function during tidal ventilation and end expiratory hold (within subject factors breathing and PEEP). The effect of static inspiratory pressure on venous return function at the two PEEP levels

316 was assessed using analysis of variance for repeated measures (within subjects  
317 factors  $P_{\text{insp}}$  and PEEP level). The effect of  $P_{\text{insp}}$  on venous return function was  
318 assessed separately in each volume state, and compared between the volume states  
319 at each  $P_{\text{insp}}$  using analysis of variance for repeated measures. The effect of  
320 inspiratory holds on blood flow decrease and restoration was analyzed using  
321 repeated measures analysis of variance (for vena cava within subject factors vessel  
322 and  $P_{\text{insp}}$ , for  $Q_{\text{PA}}$  flow pattern and  $P_{\text{insp}}$ ; PEEP and volume state as grouping factors).  
323 All data are shown as mean (SD).  
324

## RESULTS

Of the 10 animals studied, one died due to rupture of the right atrium and superior vena cava before the first set of measurements and a second animal developed prolonged ventricular fibrillation before measurements at *euvolemia* were completed. Hence, 42 of the planned 50 MSFP<sub>RAO</sub> measurements could be performed. The inflation of the right atrial balloon resulted in an abrupt cessation of PA blood flow, verified as disappearance of the PA pressure pulsatility (Figure 2). All 42 occlusions could be maintained for 60 seconds, and the hemodynamics were rapidly restored after deflation of the atrial balloon.

*1) Do changes in PEEP, volume state and tidal breaths alter MSFP and the slope of the venous return curve?*

At PEEP 10 cm H<sub>2</sub>O as compared to PEEP 5 cm H<sub>2</sub>O, both RAP and MSFP<sub>RAO</sub> increased, but RAP increased more than MSFP<sub>RAO</sub> so that VRdP decreased and RVR did not change.  $\Delta$ RAP<sub>tm</sub> did not change between PEEP levels. (Table 1, Figure 5a). Acute bleeding reduced MSFP<sub>RAO</sub> more than RAP, and hence, VRdP and Q<sub>PA</sub> decreased. Hypervolemia increased MSFP<sub>RAO</sub> more than RAP, and VRdP and Q<sub>PA</sub> increased relative to their euolemia levels. The volume state had a significant effect on  $\Delta$ RAP<sub>tm</sub>. Bleeding and hypervolemia did not change RVR (Figure 5b). The relationship between VRdP and Q<sub>PA</sub> over the volume states was highly linear (Figure 6).

RAP increased and Q<sub>PA</sub> decreased with tidal breathing slightly but significantly in all study conditions when compared to an expiratory hold (Figure 7 a and b). There was a small decrease in RVR with tidal breathing in all volume states (Figure 7 b).

348 The  $C_{\text{vascular}}$ , was  $3.2 (.7) \text{ mL} \times \text{mmHg}^{-1} \times \text{kg}^{-1}$ . The respective  $V_s$  before bleeding was 42  
349  $(9) \text{ mL} \times \text{kg}^{-1}$ , or 43 (10) % of the total blood volume.

350 *2) Does  $MSFP_{\text{insp\_hold}}$  correspond to  $MSFP_{\text{RAO}}$ ?*

351 Three of the  $MSFP_{\text{insp\_hold}}$  assessments had to be discontinued due to hemodynamic  
352 instability (in two animals: one at PEEP 10 cm  $\text{H}_2\text{O}$ , two after bleeding), and two were  
353 excluded due to lack of a sufficient linear fit. Paired comparisons - possible for 37  
354 measurements of  $MSFP_{\text{RAO}}$  - showed that  $MSFP_{\text{insp\_hold}}$  was significantly higher than  
355  $MSFP_{\text{RAO}}$  [ $16.5 (5.8) \text{ mmHg}$  vs.  $13.6 (3.2) \text{ mmHg}$ ;  $p=.001$ ; mean difference  $3.0 (5.1)$   
356  $\text{mmHg}$  for all paired measurements; Table 2]. The VRdP and RVR based on  
357  $MSFP_{\text{insp\_hold}}$  were both higher than  $MSFP_{\text{RAO}}$ -based values ( $p<.001$  and  $p=.003$ ,  
358 respectively).

359 *3) Do inspiratory hold maneuvers modify the hemodynamic variables of venous*  
360 *return function, and does PEEP and volume status modify these responses?*

361 At both PEEP levels the  $Q_{\text{PA}}$  and RAP obtained during inspiratory holds shifted to the  
362 right from the reference venous return curve based on the  $MSFP_{\text{RAO}}$ . This was not  
363 the case after *bleeding* and in *hypervolemia* (Figure 8 a and b, Table 2).

364

365 The inspiratory hold maneuvers produced a rapid initial decrease of  $Q_{\text{PA}}$ , which  
366 partially recovered during sustained hold (Figure 4 and Table 3). The  $Q_{\text{PA}}$  nadir was  
367 reached during the first two cardiac cycles after starting the inspiratory hold, and the  
368 respective nadirs of the vena cava flows occurred during the preceding cardiac cycle.  
369 The maximum decrease in blood flow was different between the IVC and the SVC  
370 and modified by the PEEP level and the  $P_{\text{insp}}$  (Table 4 and 5). Overall, the  $Q_{\text{IVC}}$   
371 decreased more than the  $Q_{\text{SVC}}$  and was lowest after *bleeding*. This difference

372 between the vessels was most prominent at *PEEP 5 cm H<sub>2</sub>O* and *euvolemia* (Table 4  
373 and 5).

374 The  $Q_{IVC}$  recovered most at lowest inspiratory pressures independent of volume state  
375 and more than the  $Q_{SVC}$  did (Table 4). The recovery occurred before the time point  
376 used to estimate  $MSFP_{insp\_hold}$ . There were no significant differences between the  
377  $Q_{IVC}$  and  $Q_{SVC}$  in the maximum decrease or in the recovery from  $Q_{PA}$  nadir after  
378 bleeding and in hypervolemia.

379 The inspiratory hold maneuvers led to a progressive and linear increase in the  
380 inspiratory hold induced changes in transmural pressure of the SVC, indicating that  
381 transmural pressure became progressively lower with increasing plateau pressure  
382 (Figure 9).

383

## DISCUSSION

The main findings of our study were

1) Increased PEEP during positive pressure ventilation with moderate tidal volumes produced an increase in MSFP, which almost completely compensated for the concomitant increase in RAP and did not change RVR. Consequently, cardiac output did not change. When blood volume was altered, MSFP and RAP changed in the same direction, but RAP was less affected. Accordingly, VRdP and cardiac output decreased and increased in parallel with blood volume, but again RVR did not change.

2)  $MSFP_{insp\_hold}$  overestimated the  $MSFP_{RAO}$  in euvoletic conditions, regardless of the PEEP level, whereas in *bleeding* and *hypervolemia* the observed values were very similar.

3) The inspiratory hold maneuvers shifted the venous return pressure/flow relationship to the right of the reference venous return curve in euvoletic conditions but did not do so in bleeding or hypervolemia.

In order to explain the shift of the pressure/flow relationship during the inspiratory holds and the consequent overestimation of  $MSFP_{RAO}$  by  $MSFP_{insp\_hold}$  during euvoletic conditions but not with bleeding or hypervolemia, we analyzed the time course of changes in both caval and pulmonary artery flows.  $Q_{PA}$  decreased initially very rapidly but was then partially recovered (see Table 3). As expected, the nadir of vena cava flows preceded that of the  $Q_{PA}$  by one cardiac cycle but the patterns of decrease and recovery of blood flows differed between the IVC and the SVC in euvoletic

conditions. Although  $Q_{IVC}$  initially decreased more than  $Q_{SVC}$ , it recovered more completely in the euvoletic condition but not in bleeding or hypervolemia, and thus changes in caval flow during recovery patterns matched the shifts of the pressure/flow relationship in the three conditions. This recovery in  $Q_{IVC}$  occurred very rapidly within a few heartbeats, making reflex activation unlikely, and indicates that there must have been other adaptive mechanisms in the vascular compartments drained by the IVC. This flow recovery we observed cannot be explained by Guyton's model.

Two distinct mechanisms control hepatosplanchnic blood flow, when the outflow pressure is increased depending upon the venous pressure. The hepatic drainage has a vascular waterfall – or Starling resistor - that can be overcome when the outflow pressure is greater than 5 mmHg which will change the pressure/flow relationship (4, 9, 50). At higher outflow pressures, liver venous resistance decreases, consistent with passive distention of the venous system (9). In isolated porcine liver, distensibility appears to be maximal at outflow pressures above 10 mmHg (9). We have previously shown in intensive care patients that the venous driving pressure across the liver does not change in response to a 5 cm H<sub>2</sub>O PEEP increase from 7-11 cm H<sub>2</sub>O (range in individual patients) to 10-14 cm H<sub>2</sub>O (34). Thus, these two mechanisms could act in concert to defend the hepatosplanchnic and IVC venous return. When RAP acutely increases, first the waterfall/Starling resistor is overcome and then there is distention of the vessels. These compensations would not occur in the hypervolemia condition because the higher RAP would have overcome the resistor and the drainage would already be maximally distended. Portal venous pressure equilibration with MSFP is delayed up to at least seven seconds in

hypovolemic conditions (20), and provides additional drainage of the splanchnic compartment. A waterfall with collapse in the IVC, as shown by Fessler in dogs (16), may provide an additional mechanism. Vessel compression has been shown to occur in the SVC during mechanical ventilation and it is accentuated in hypovolemia (74, 75). We show now indirect evidence of a progressive compression of the SVC with the inspiratory hold maneuver. The linear relationship between changes in vessel flow and transmural pressure in the studied range of airway pressures (Figure 9) suggests vessel closure if airway pressures would be further increased. Since the relationship between transmural pressure change and blood flow change may not be linear once the vessel is close to collapse (52), we cannot reliably estimate the critical closing pressures. For caval vein closure, higher transduction of airway or pleural pressure to the SVC than to the right atrium must be present. This has been shown by Fessler (16) and later in patients by Lansdorp (35). Our data show unchanged transmural atrial pressure between PEEP levels, which further supports this possibility.

A fourth mechanism that may contribute is the hepatic arterial buffer response. It increases the hepatic arterial flow acutely, when portal venous flow decreases. Low systemic blood flow reduces the hepatosplanchnic blood flow and partially abolishes these compensation mechanisms (30). In hypervolemia, the increased RAP would be expected to reduce the hepatic blood flow defense by exceeding the waterfall and by approaching the limits of the distensible system. Thus there was no shift in  $MSFP_{insp\_hold}$ . Since we did not measure hepatic blood flow, these proposed mechanisms need further confirmation. Venous return via the azygos vein directly into the right atrium was not accounted for, but may also have contributed to the flow recovery (26, 30). A fifth possible mechanism is an on-going shift of volume from the



arterial to venous compartments during the inspiratory hold. This seems unlikely as the sole explanation, since the volume shift necessary to explain the mean difference between  $MSFP_{RAO}$  and  $MSFP_{insp\_hold}$  would be in the range of 300 mL and would have to occur within seconds. The volume transfer from the arterial to the venous tree due to elastic recoil during circulatory standstill has been estimated to be around 4 mL/kg (64).

Regardless of the mechanism, volume status modified the shift of the  $Q_{PA}/RAP$ . The main interest in estimation of MFSP in the clinical setting is to understand better the complex hemodynamic problems and the response to therapeutic interventions. Our results clearly demonstrate that  $MSFP_{RAO}$  and  $MSFP_{insp\_hold}$  are not interchangeable and that  $MSFP_{insp\_hold}$  overestimates the  $MSFP_{RAO}$ . The impact of volume status on the  $Q_{PA}/RAP$  in our model of healthy anesthetized pigs was quantitatively moderate. Overall, our values obtained with the balloon occlusion method are in the same range as others have obtained in pigs (53) and dogs (51) with the same method, and are also close to the MSFP of ICU patients promptly after death, as reported by Vieillard-Baron and co-workers (62). In contrast, the MSFP values obtained with the inspiratory hold method in postoperative and septic patients are considerably higher (42, 57). Our results provide a possible mechanism for such unexpectedly high MSFP values. Since considerably larger volume shifts than in our study are common in patients with hemodynamic problems, shift of  $Q_{PA}/RAP$  during inspiratory holds may be more pronounced. The  $MSFP_{insp\_hold}$  values exceeding 30 mmHg reported in septic patients (57) may at least in part be explained by the direct physiologic effects of inspiratory holds.

Before criticizing our methodology, the conceptual issues related to the interpretation need to be discussed. The physiologic relevance and the actual existence of MSFP during on-going blood flow has been heavily debated. The MSFP is not located in any particular subdivision, but represents the stressed volume of the entire systemic vasculature. We consider it as the weighted mean of elastic recoil pressures in all systemic vascular beds, as measured after venous pressure equilibration during zero flow induced by RA occlusion. It will change if volume shifts alter the stressed volume, or if vascular elastance changes. When venous return is reduced during an inspiratory hold, volume will increase in the systemic vascular compartment due to reduced outflow and sustained inflow, until a new steady state has been reached(73). The low elastance compartment will receive most of this volume shift. Since the pulmonary circulation and the heart contribute to the volume shift, the stressed volume of the systemic circulation will increase, and consequently also the elastic recoil pressure caused by the stressed volume, i.e. the MSFP. The increase in MSFP due to such volume shifts in our experimental conditions would be very small - around 1-2 % (data not shown) and consistent with the result from other groups (73). Such volume shifts could therefore not account for the observed differences between  $MSFP_{RAO}$  and  $MSFP_{insp\_hold}$ .

A second important issue is whether RA pressure acts as back pressure to venous return, or whether it only responds passively to volume shifts when flow changes, as proposed by Levy and Brengelmann (8, 36). Our study was not designed to solve this central point in the debate between proponents of Guyton and those of Levy. As discussed by Tyberg (69), *"It must be acknowledged that both interpretations are model-based and both are internally consistent. Thus, it is very difficult or perhaps impossible to 'prove' one at the expense of the other"*.

507 The observed linearity of the RAP/ $Q_{PA}$  relationship during the inspiratory hold  
508 maneuvers is compatible with both Levy's and Guyton's models, while neither would  
509 *a priori* predict the occurrence of Starling resistors/waterfalls(73) or flow recovery  
510 situations as we describe them.

511 There are some important methodological limitations to our study. Since we only  
512 measured the  $MSFP_{RAO}$  during expiratory holds, it is possible that MSFP changes  
513 during the respiratory cycle. We tried to address this by plotting the  $Q_{PA}/RAP$  during  
514 tidal breathing and end expiratory hold with the  $MSFP_{RAO}$  (Figure 7). These venous  
515 return curves were almost superimposed. However, we observed a very small but  
516 significant decrease in RVR during tidal breathing as compared to expiratory hold –  
517 i.e. the  $Q_{PA}$  and RAP during tidal breathing shifted slightly down and to the right  
518 (Figure 7). It is unlikely that compliance changes could occur during one breath (60).  
519 An alternative explanation for this apparent change in RVR is that tidal inflations may  
520 enhance volume shifts from the pulmonary circulation, and therefore increase the  
521  $MSFP_{RAO}$  without changing RVR (10). Volume shifts in pulmonary blood volume  
522 during mechanical ventilation are small and depend on the zone conditions of the  
523 lung (10). Given the large  $C_{vascular}$  in our experiment, the effect on MSFP would be  
524 negligible. To further assess the behavior of MSFP during mechanical ventilation,  
525  $MSFP_{RAO}$  at expiratory hold and tidal breathing should be compared in future studies.

526 The balloon obstruction of the right atrium for determination of MSFP (51) is likely to  
527 result in slightly higher values than those obtained using ventricular fibrillation, since  
528 the beating heart shifts some volume from the pulmonary to systemic circulation(63).  
529  $MSFP_{RAO}$  therefore dissociates from mean circulatory filling pressure obtained after  
530 instantaneous cardiac arrest and full equilibration of all intravascular pressures. This

531 difference is likely to be marginal, since previous comparisons of balloon derived  
532 MSFP with potassium induced cardiac arrest showed no difference(53).

533 Furthermore, we did not use reflex blockade in our experiment, since it is not relevant  
534 for the clinical application of MSFP estimation. Anesthetic drugs may therefore have  
535 influenced the measurements.

536 Previous studies on the effects of increased intrathoracic pressure and positive  
537 pressure ventilation on venous return function have provided controversial results.  
538 Fessler et al. found that a PEEP of 15 cm H<sub>2</sub>O vs no PEEP had no impact on VRdP  
539 in a ventricular fibrillation canine model, and since cardiac output decreased, the  
540 RVR had to increase (18). The increase in resistance to venous return with PEEP  
541 was confirmed with a venous bypass preparation (17). A brief change of airway  
542 pressure from 0 to 15 cm H<sub>2</sub>O during apnea and ventricular fibrillation raised MSFP  
543 to the same extent as RAP was increased by apneic airway pressure. Since cardiac  
544 output decreased in response to higher airway pressure, the RVR must have  
545 increased (33). Nanas and Magder also found that increasing PEEP from 0 to 20 cm  
546 H<sub>2</sub>O had no effect on VRdP, but increased RVR (51). Changing from spontaneous  
547 breathing to positive pressure ventilation in rats increased the MSFP and decreased  
548 the VRdP and cardiac output without an effect on RVR (13).

549

550 In contrast to previous studies, we found no change in RVR in response to PEEP.  
551 The differences in experimental setting and the measurement of variables of RVR,  
552 that is RAP, MSFP, and cardiac output, should be considered in interpreting the  
553 results. Most previous studies used much higher airway pressure changes and larger  
554 tidal volumes, 12-15 as compared to 7-8 mL/kg in the present study (13, 17, 18, 51).  
555 Furthermore, we used the same airway plateau pressures during the inspiratory hold

maneuvers with lower and higher PEEP. Accordingly, only PEEP and end expiratory lung volume were increased, resulting in lower airway driving pressure at the higher PEEP. This is likely to explain the modest effects of PEEP on cardiac output. In addition, we have previously shown in intensive care patients that a 5 cm H<sub>2</sub>O PEEP increase from 7-11 cm H<sub>2</sub>O (range in individual patients) to 10-14 cm H<sub>2</sub>O has no effect on cardiac output (34). The mechanical effects of high PEEP with high tidal volumes on venous return, right heart function, and pulmonary vasculature are likely to be very different from our approach, and may explain much of the seemingly controversial results. On-going tidal positive pressure breathing (18), or turning ventilator off but not in a specific point of breath (51) may also modify the response. It is conceivable that on-going inflations during the assessment of MSFP may enhance volume shift from the pulmonary circulation (10), and therefore increase the MSFP to values higher than during normal circulation. This would result in an apparently higher RVR.

Our results on effects of PEEP cannot be extrapolated to conditions where higher PEEP levels are commonly used, such as acute lung injury. However, transmission of higher airway pressures to pleural pressures in acute lung injury may be attenuated due to impaired lung compliance (32).

Despite the higher MSFP estimates with the inspiratory hold method, the  $Q_{PA}/RAP$  response to the transient changes appeared remarkably linear, still consistent with Guyton's model. This suggests that despite the perturbations, a new steady state with a new resultant MSFP is achieved rapidly.

The further issue is the measurement of cardiac output as surrogate of venous return. Most previous studies on the effects of PEEP have used intermittent transcardiac thermodilution measurements (13, 18, 51), whereas we measured  $Q_{PA}$

beat-by-beat, which allowed us to evaluate the  $MSFP_{RAO}$  with the pressure and flow measurements right before the balloon occlusion. A similar approach was used in a landmark description of right ventricular heart lung interactions (59) and in the initial description of inspiratory holds for estimation of  $MSFP_{insp\_hold}$  (73). The estimations of  $MSFP_{RAO}$  in critical care patients have used arterial pulse contour analysis (37, 39-42, 57). Cardiac output measured by arterial pulse contour analysis does not track acute changes in venous return during brief periods because of the time delay between the change in outputs of the right and left ventricles. This is caused by buffering by the pulmonary vasculature and the transit time for rightsided changes to reach the left side during the respiratory cycle (10, 65, 72).

In conclusion, we found that during positive pressure ventilation with moderate levels of PEEP and low tidal volumes consistent with current recommended clinical practice (1, 66), PEEP produced modest changes in venous return, which were due to changes in VRdP and without alterations in RVR. This indicates that the concepts of mechanisms by which PEEP modifies hemodynamics should be revised when low tidal volumes and airway driving pressures are used. Furthermore, we conclude that inspiratory holds alter the venous pressure flow relationships, so that their use for bedside assessment of MSFP may be misleading and needs to be further studied.

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607

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621

622 **Authors Contributions**

623

624 DB: conception of study and design of the protocol, preparation and performance of  
625 the experiment, data analysis and interpretation, drafting and revisions of the  
626 manuscript

627 PWM: contribution to the protocol design, performance of the experiment, data  
628 analysis and interpretation, drafting and revisions of the manuscript

629 AW: conducted the cardiac surgery, contribution to and revision of the manuscript

630 AB: performance of the experiment and revision of the manuscript

631 SB: preparation and performance of the experiment and revision of the manuscript

632 MH: protocol design, preparation and performance of the experiment, revision of the  
633 manuscript

634 SS: protocol design, data interpretation, revision of the manuscript

635 SMJ: conception of study, data interpretation, revision of the manuscript

636 SM: data analysis and interpretation, drafting and revision of the manuscript

637 JT: conception of study, data analysis including statistics and data interpretation,  
638 drafting and revising the manuscript. Study sponsor

639 All authors approved the final version of the manuscript

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## 840 **Figure Captions**

841 **Figure 1:** The course of the experimental protocol is depicted. The experiment was  
842 divided in two parts. Part A examined the effects of changes in positive end  
843 expiratory pressure on the venous return function. Part B assessed the effects of  
844 volume changes.

845 **Figure 2.** Time course of intravascular pressures after balloon occlusion (black  
846 arrow) of the right atrium. The mean of pressures from 9 to 12 seconds after  
847 occlusion were used to estimate the  $MSFP_{RAO}$ . Sympathetic activation is apparent as  
848 an increase in all pressures approximately 10 seconds later.

849 **Figure 3:** An exemplary extrapolation of stressed and unstressed volume is shown.  
850 Blood volume was measured at *Euvolemia* with ICG, see methods. After bleeding  
851 and retransfusion,  $MSFP_{RAO}$  was measured. Stressed volume could be extrapolated  
852 with a linear regression (40, 51). The slope of the line equals the inverse of vascular  
853 compliance (elastance). Equation for the above graph,  $MSFP = -19.939 + (0.0077 \times$   
854  $Blood\ Volume), r^2 = 0.988., C_{vascular} = 129.9\ mL/mmHg, 3.2\ mL \times mmHg^{-1} \times kg^{-1}.$   
855  $V_u$ :unstressed volume,  $V_s$ :stressed volume.

## 856 **Figure 4:**

857 The figure describes the inspiratory hold maneuvers and their analysis. Expiratory  
858 holds at the given PEEP and inspiratory holds at plateau pressures of 15, 20 and 25  
859 cm H<sub>2</sub>O were performed over 30 seconds at all experimental conditions (Panel A).

860 *Extrapolation of  $MSFP_{insp\_hold}$ :* Mean values for  $Q_{PA}$  and RAP of the first three cardiac  
861 cycles occurring 9 seconds into the maneuver (green shade, Panel B and C) were  
862 taken and extrapolated to zero flow in order to estimate  $MSFP_{insp\_hold}$  (Panel D).

*Flow behavior in the thoracic veins:* Inspiration causes an immediate drop in flow, visible as a single nadir beat for the caval veins (red shade in Panel B and C), transmitted to the pulmonary artery in the next heartbeat. Partial restoration of flow can be observed during the following beats. To assess these dynamic flow changes, the mean for all heartbeats during a full respiratory cycle preceding the inspiratory hold is used as *baseline* (blue shade Panel B and C). *Flow decrease* is presented as ratio of the nadir beat (red shade) to baseline (blue shade). *Flow restoration* is presented as the ratio of mean flow during the three beats at 9 seconds (green shade) to *baseline* (blue shade).

**Figure 5:** Effect of PEEP (Panel A) and acute alterations in blood volume (Panel B) on venous return function. Right atrial pressure and pulmonary artery blood flow were measured during tidal breathing for 10 cardiac cycles and the  $MSFP_{RAO}$  as the mean of the caval pressures for 3 seconds at zero flow 9 seconds after right atrial balloon occlusion at end-expiratory lung volume. The expiratory hold was started immediately before the right atrial balloon was filled. The lines connect the mean values, while RVR was calculated in every individual animal, for details see Table 1. Effect of PEEP:  $MSFP_{RAO}$   $p=.002$ , RAP:  $p<.001$ ,  $Q_{PA}$   $p=.094$ ; effect of volume:  $MSFP_{RAO}$   $p<.001$ ; RAP:  $p<.001$ ,  $Q_{PA}$   $p<.001$ ; Values are shown as means, error bars indicate one standard deviation.

**Figure 6:** Linear regressions were done over the three volume states for venous return driving pressure VRdP ( $=MSFP - RAP$ ) and  $Q_{PA}$ . The relationship is highly linear with a median  $r^2$  of 0.976 (range 0.726 - 1).

**Figure 7.** Effect of tidal ventilation on venous return function. Right atrial pressure and pulmonary artery blood flow was measured for 10 cardiac cycles during tidal breathing and during an expiratory hold immediately before the inspiratory hold maneuvers. The respective right atrial pressure and pulmonary artery blood flow values were plotted with the  $MSFP_{RAO}$  to show the venous return during tidal breathing and at end-expiratory lung volume. Values are shown as means, error bars indicate one standard deviation. TV: tidal ventilation, exp: end expiration.

*Solid line:* end expiration; *dotted line:* tidal ventilation

**a.)** PEEP 5 cm H<sub>2</sub>O and PEEP 10 cm H<sub>2</sub>O

Effect of tidal ventilation: RAP  $p < .001$ ,  $Q_{PA}$   $p < .001$ , RVR  $p = .161$

**b.)** euvoemia, bleeding, hypervolemia

effect of tidal ventilation RAP:  $p < .001$ ,  $Q_{PA}$   $p < .001$ , RVR  $p < .001$

**Figure 8:** Venous return at end-expiratory lung volume and inspiratory holds. Right atrial pressure and pulmonary artery blood flow was measured over three cardiac cycles from 9 seconds into each expiratory and inspiratory hold and plotted with the  $MSFP_{RAO}$  of each condition. The  $Q_{PA}$  and the corresponding RAP during inspiratory holds for each individual animal were used to construct individual linear regression lines. Their zero flow intercepts represent the  $MSFP_{insp\_hold}$  for each animal and study condition, details see methods and figure 4. Values at expiratory hold values and the respective  $MSFP_{RAO}$  were used as the reference venous return function. Values are shown as means, error bars indicate one standard deviation.

a.) PEEP 5 cm H<sub>2</sub>O (triangle upwards) and PEEP 10 cm H<sub>2</sub>O (triangle downwards), grey scale indicating increasing airway plateau pressure:

Statistics:

912 effect of PEEP: RAP  $p=.037$ ,  $Q_{PA}=.713$

913 effect of  $P_{insp}$ : RAP  $p<.001$ ,  $Q_{PA} p<.001$

914  $P_{insp}$  \*PEEP interaction: RAP  $p=.031$ ,  $Q_{PA} p=.020$

915

916 b.) Euvolemia (circle), bleeding (square), hypervolemia (diamonds), grey scale

917 indicating increasing airway plateau pressure:

918 Statistics:

919 effect of  $P_{insp}$ : in all volume states, RAP  $p<.001$ ,  $Q_{PA}$ : euvolemia  $p<.001$ ,

920 bleeding  $p=.001$ , hypervolemia  $p<.001$

921 effect of volume state: at all pressure levels: RAP  $p<.001$ ,  $Q_{PA} p<.001$

922

923 **Figure 9.** Respiratory changes in transmural pressure of the SVC were analyzed

924 over the inspiratory hold manoeuvres. With increasing plateau pressure, the change

925 became progressively and linearly more negative, suggesting vessel compression.

926 The linear regression equations are

927 for PEEP 5 cmH<sub>2</sub>O:  $\Delta P_{tm} = -2.352 + (0.00325 \times Q_{SVC})$ ,  $r^2 = 0.98$

928 for PEEP 10 cm H<sub>2</sub>O:  $\Delta P_{tm} = -1.839 + (0.00331 \times Q_{SVC})$ ,  $r^2 = 0.855$

929

930



931 **Tables**

932 **Table 1. Effect of PEEP and blood volume on hemodynamics**

	PEEP 5 cmH <sub>2</sub> O (n=9)	PEEP 10 cmH <sub>2</sub> O (n=9)	<i>p</i>	Euvolemia PEEP 5 cmH <sub>2</sub> O (n=8)	Bleeding PEEP 5 cmH <sub>2</sub> O (n=8)	Hypervolemia PEEP 5 cmH <sub>2</sub> O (n=8)	<i>p</i>
Heart rate; beats/min	100 (29)	96 (23)	.685	102 (21)	129 (31)	106 (20)	.001
MAP; mmHg	63 (7)	61 (12)	.609	60 (10)	50 (11)	63 (12)	.012
PAP; mmHg	18 (3)	20 (3)	.018	19 (3)	17 (3)	23 (3)	<.001
RAP; mmHg	5.9 (1.6)	7.5 (1.4)	<.001	5.9 (1.6)	5.1 (1.7)	8.2 (1.9)	<.001
$\Delta$ RAP <sub>tm<sub>exp</sub></sub> ; mmHg	.26 (1.02)		.496	-	.29 (.62)	.98 (1.26)	.033
Q <sub>PA</sub> ; L/min	2.75 (.43)	2.56 (.45)	.094	2.80 (.46)	2.20 (.42)	3.27 (.42)	<.001
MSFP <sub>RAO</sub> ; mmHg	12.9 (2.5)	14.0 (2.6)	.002	13.0 (2.8)	10.8 (2.2)	16.4 (3.0)	<.001
VRdP; mmHg	7.0 (2.2)	6.5 (2.3)	.033	7.0 (2.4)	5.7 (1.7)	8.2 (2.2)	<.001
RVR; mmHg/L/min	2.53 (.52)	2.53 (.63)	.945	2.49 (.59)	2.60 (.58)	2.50 (.52)	.489
	before PEEP changes			before bleeding	after bleeding	in hypervolemia	
Blood volume*; mL/kg	96 (14)			98 (16)	89 (15)	113 (21)	.008

933 MAP=mean arterial pressure; PAP=mean pulmonary artery pressure; RAP=right atrial pressure;  $\Delta RAP_{tm_{exp}}$ = expiratory right atrial transmural  
 934 pressure,  $Q_{PA}$ =pulmonary artery blood flow;  
 935 mean of 10 cardiac cycles before balloon occlusion during positive pressure ventilation with a tidal volume of 300 mL [7.7(.3) mL/kg]. Values for  
 936  $\Delta RAP_{tm_{exp}}$  are differences between experimental conditions of expiratory mean values of 5 respiratory cycles before balloon occlusion of 8 pigs  
 937 (one pig excluded due to local hematoma around the pericardial balloon catheter).  
 938  $MSFP_{RAO}$ =mean systemic filling pressure; measured during right atrial balloon occlusion at end expiratory lung volume  
 939  $VRdP$ = venous return driving pressure;  $VRdP = MSFP_{RAO} - RAP$ ,  $RVR$ =resistance to venous return;  $RVR = VRdP / Q_{PA}$   
 940 \*Blood volume after bleeding calculated as volume measured before bleeding – volume of shed blood  
 941 p-values: paired t-test for PEEP effect, repeated measures analysis of variance for effect of volume status, repeated measures analysis of variance  
 942 for blood volume. Data shown for animals completing each series (PEEP n=9, volume n=8). Values are mean (SD).  
 943  
 944

945 **Table 2. Comparison of MSFP<sub>RAO</sub> and MSFP<sub>insp\_hold</sub> at different PEEP-levels and blood volumes**

	PEEP 5 cmH <sub>2</sub> O (n=8)	PEEP 10 cmH <sub>2</sub> O (n=7)	Euolemia PEEP 5 cmH <sub>2</sub> O (n=8)	Bleeding PEEP 5 cmH <sub>2</sub> O (n=6)	Hypervolemia PEEP 5 cmH <sub>2</sub> O (n=8)	<i>p</i> *
MSFP <sub>RAO</sub> ; mmHg	12.9 (2.6)	14.1 (3.0)	13.0 (2.8)	10.9 (2.6)	16.4 (3.0)	.002
MSFP <sub>insp_hold</sub> ; mmHg	15.7 (2.7)	18.7 (4.0)	15.9 (3.7)	11.9 (2.0)	19.7 (9.8)	

946 Comparison of all available paired measurements (n=37); \* p-value for repeated measures analysis of variance for effect of measurement method;  
 947 no interaction (p=.802) between method and underlying clinical condition (PEEP-level, volume status). Values are mean (SD).  
 948

949

950 **Table 3. Blood flow decrease and restoration in pulmonary artery during inspiratory holds**

	P <sub>insp</sub> 15			P <sub>insp</sub> 20			P <sub>insp</sub> 25			
	Baseline [L/min]	minimum flow [L/min]	flow restoration [L/min]	Baseline [L/min]	minimum flow [L/min]	flow restoration [L/min]	Baseline [L/min]	minimum flow [L/min]	flow restoration [L/min]	interactions with flow pattern <i>p</i>
PEEP 5 cmH <sub>2</sub> O	2.83 (.59)	2.08 (.56)	2.48 (.58)	2.77 (.53)	1.78 (.56)	2.21 (.53)	2.72 (.51)	1.37 (.59)	2.00 (.49)	PEEP <.001
										P <sub>insp</sub> <.001
PEEP 10 cmH <sub>2</sub> O	2.35 (.44)	2.02 (.50)	2.27 (.46)	2.44 (.53)	1.78 (.52)	2.13 (.50)	2.43 (.45)	1.56 (.47)	2.02 (.48)	
Euvoemia	2.60 (.58)	1.87 (.65)	2.30 (.56)	2.54 (.57)	1.41 (.62)	2.01 (.53)	2.60 (.67)	1.12 (.65)	1.82 (.50)	P <sub>insp</sub> <.001
										P <sub>insp</sub> *volume <.001
Bleeding	2.21 (.44)	1.44 (.39)	1.77 (.39)	2.27 (.43)	.73 (.46)	1.51 (.35)	1.51 (.57)	.60 (.09)	1.19 (.44)	
Hypervolemia	3.24 (.59)	2.63 (.76)	2.92 (.59)	3.12 (.50)	1.90 (.68)	2.56 (.67)	3.08 (.52)	1.63 (.69)	2.27 (.65)	

951  
 952 Statistics: **PEEP-levels:** repeated measures analysis of variance for Q<sub>PA</sub> with flow pattern (baseline, nadir, restoration) and P<sub>insp</sub> as within subject  
 953 factors and PEEP as grouping factor. The p-values indicate interaction of PEEP and P<sub>insp</sub> with flow pattern. **Volume status:** Repeated measures  
 954 analysis of variance with flow pattern (baseline, nadir, restoration) and P<sub>insp</sub> as within subject factors and volume state as grouping factor. The p-  
 955 values indicate interaction of P<sub>insp</sub> with flow pattern. Values are mean (SD).  
 956

957 **Table 4. Blood flow decrease and restoration in caval veins during inspiratory holds at different levels of PEEP**

		Maximum decrease in flow (fraction of baseline)			<i>p</i>			
		P <sub>insp</sub> 15	P <sub>insp</sub> 20	P <sub>insp</sub> 25	vessel	P <sub>insp</sub>	PEEP	interactions *
PEEP 5	IVC	0.47 (.22)	0.38 (.20)	0.04 (.21)	.002	<.001	.012	vessel*P <sub>insp</sub> *PEEP 0.037
	SVC	0.65 (.25)	0.47 (.28)	0.37 (.18)				
PEEP 10	IVC	0.74 (.15)	0.57 (.14)	0.34 (.23)				
	SVC	0.74 (.15)	0.64 (.18)	0.40 (.19)				
		Flow restoration (fraction of baseline)						
PEEP 5	IVC	0.95 (.13)	0.86 (.07)	0.78 (.09)	.013	<.001	.028	--
	SVC	0.87 (.08)	0.81 (.10)	0.75 (.19)				
PEEP 10	IVC	1.03 (.08)	0.93 (.06)	0.90 (0.9)				
	SVC	0.94 (.05)	0.89 (.09)	0.84 (.13)				

958 Blood flow changes as fraction of the respective flows during one breath cycle preceding the inflation (baseline). **The maximum decrease** is for  
959 the single nadir beat during the inspiratory hold; **the flow restoration** during the inspiratory hold is the fraction of the three beats used to  
960 extrapolate the MSFP<sub>insp\_hold</sub> of the baseline breath. Statistics: Repeated measures analysis of variance with vessel and P<sub>insp</sub> as within subject  
961 factors and PEEP as a grouping factor. Post hoc tests within each PEEP level; repeated measures analysis of variance with vessel and P<sub>insp</sub> as  
962 within subject factors. All values mean (SD); n=8

963

964 \* significant interactions, if present, are reported with the highest number of interacting variables.

965     **Table 5. Blood flow decrease and restoration in caval veins during inspiratory holds at different blood volumes**

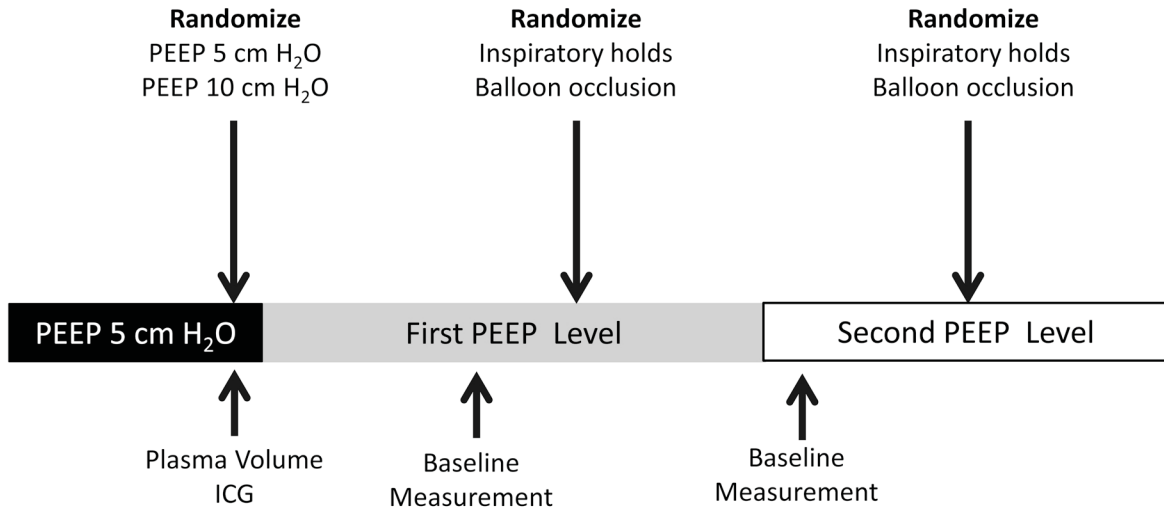
		Minimum flow (fraction of baseline)			
		P <sub>insp</sub> 15	P <sub>insp</sub> 20	P <sub>insp</sub> 25	<i>p</i>
Euvoemia (n=9)	IVC	0.43 (.21)	0.19 (.19)	0.16 (.40)	vessel .003  P <sub>insp</sub> <.001  vessel* P <sub>insp</sub> <.001  volume .057
	SVC	0.64 (.23)	0.48 (.24)	0.48 (.22)	
Bleeding (n=6)	IVC	0.36 (.24)	0.07 (.22)	-0.11 ( .32)	
	SVC	0.55 (.16)	0.33 (.16)	0.17 (.22)	
Hypervolemia (n=8)	IVC	0.58 (.10)	0.23 (.23)	0.14 (.33)	
	SVC	0.65 (.15)	0.48 (.28)	0.39 (.29)	
		Flow restoration (fraction of baseline)			
Euvoemia (n=9)	IVC	0.93 (.08)	0.79 (.12)	0.75 (.13)	vessel <.001  P <sub>insp</sub> <.001  volume .003
	SVC	0.86 (.06)	0.77 (.10)	0.70 (.09)	
Bleeding (n=6)	IVC	0.80 (.07)	0.69 (.07)	0.55 (.12)	
	SVC	0.77 (.07)	0.62 (.12)	0.51 (.17)	
Hypervolemia (n=8)	IVC	0.91 (.06)	0.81 (.08)	0.75 (.14)	
	SVC	0.90 (.10)	0.85 (.16)	0.77 (.17)	

966

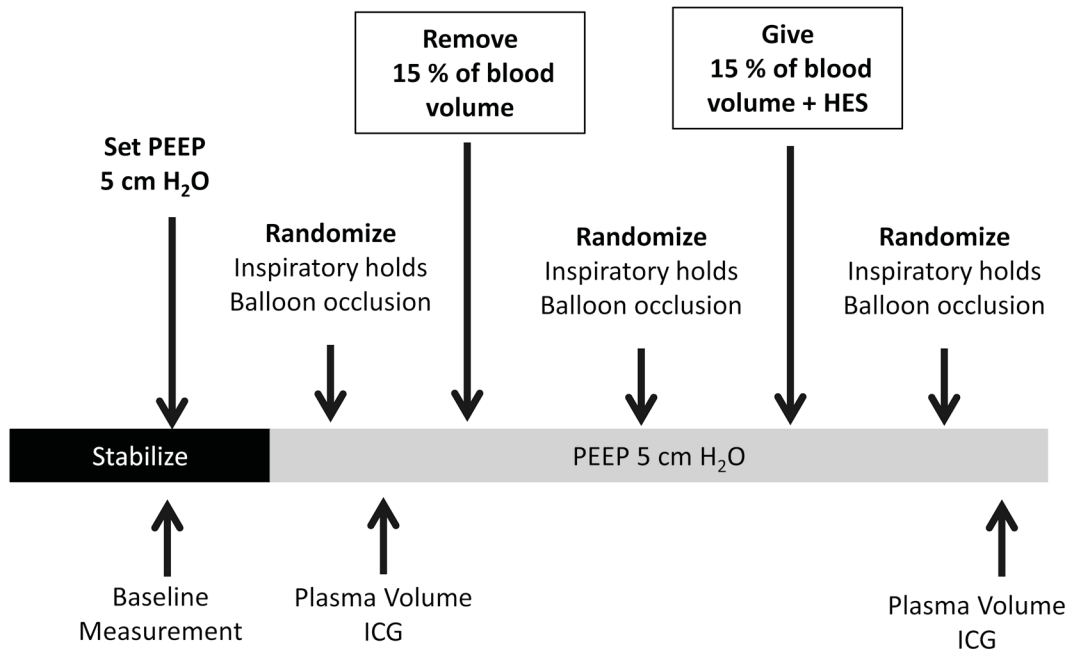
967 Blood flow changes as fraction of the respective flows during one breath cycle preceding the inflation (baseline). **The minimum flow** is for the  
968 single nadir beat during the inspiratory hold; **the flow restoration** during the inspiratory hold is the fraction of the three beats used to extrapolate  
969 the  $MSFP_{\text{insp\_hold}}$  of the baseline breath. Statistics: repeated measures analysis of variance with vessel and  $P_{\text{insp}}$  as within subject factors and  
970 volume state as grouping factor. All values are mean (SD)  
971  
972

**A**

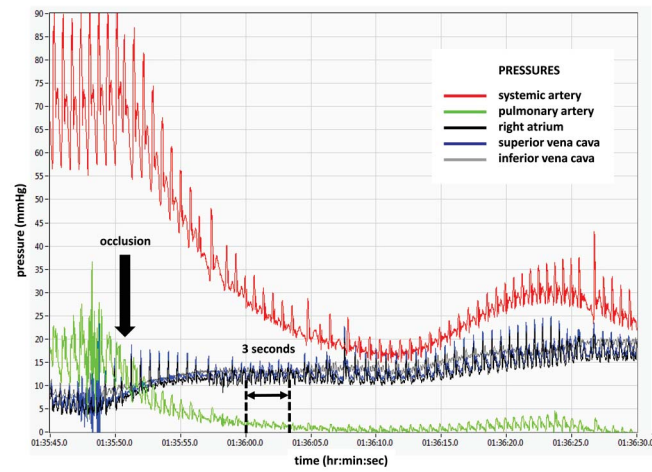
### Influence of PEEP

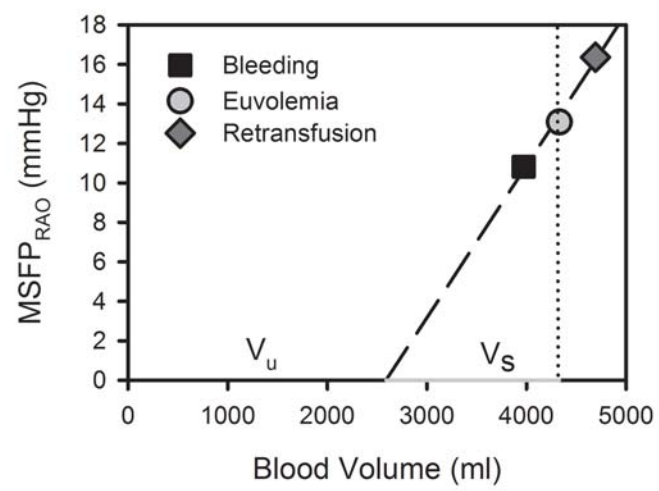
**B**

### Influence of Volume Changes

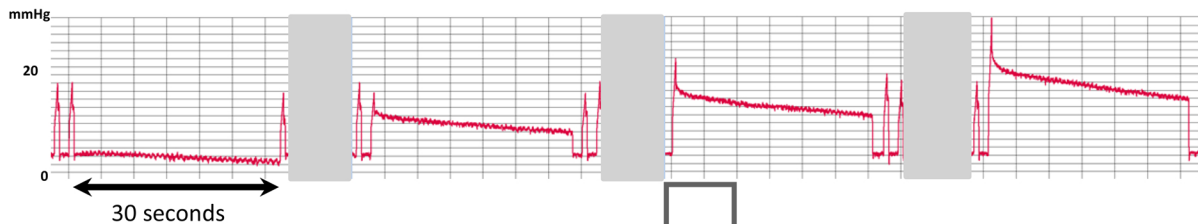




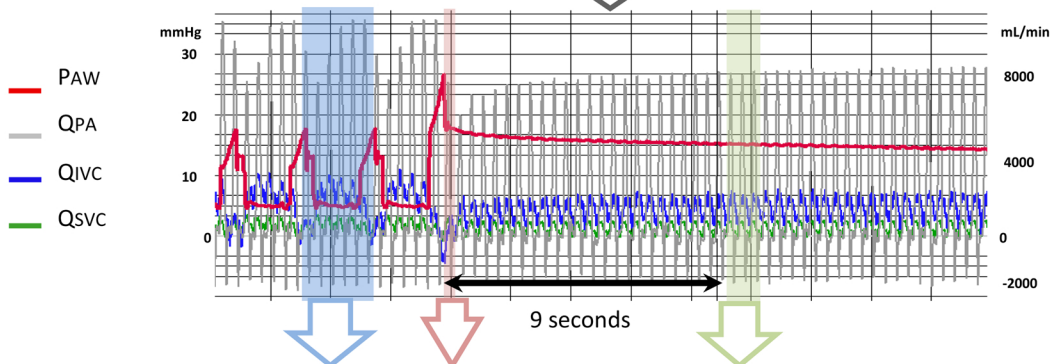




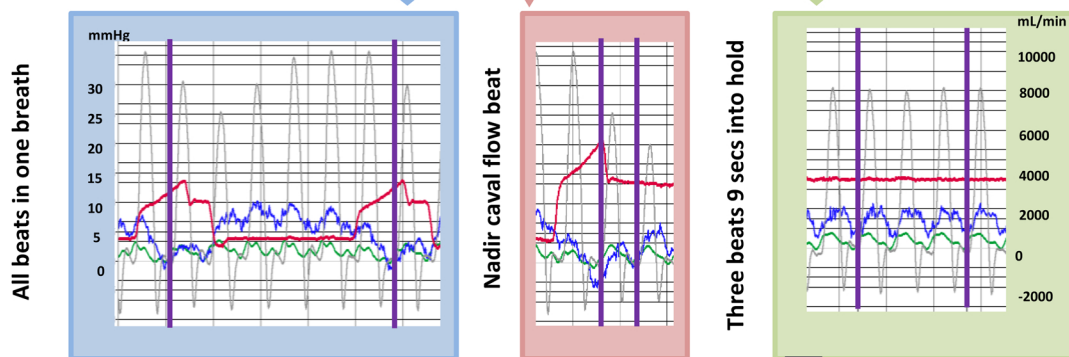
A



B



C



D

